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PENSOFT

Carolinensis minutus (Dujardin, 1845) Travassos 1937 (Nematoda, Heligmonellidae) in *Microtus agrestis* in the United Kingdom

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Abstract

The heligmonellid nematode *Carolinensis minutus* (Dujardin, 1845) Travassos, 1937 is recorded for the first time in *Microtus agrestis* (Linnaeus, 1761) in the United Kingdom. Small heligmosomoid specimens were recovered from a *M. agrestis* vole inhabiting mole (*Talpa europaea* Linnaeus, 1758) tunnels in mid Wales. The identity of these specimens was confirmed as *C. minutus* by >99% nucleotide identity with internally transcribed spacer (ITS) 1 and ITS 2 sequences in French *C. minutus*.

Keywords

Heligmosomoidea, Field Vole, rodent, European Mole, mole tunnel, transcriptome

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Introduction

Dujardin (1845) described *Carolinensis minutus* (Dujardin, 1845) Travassos, 1937, as *Strongylus minutus*, parasitic in rodents in the Rennes area of northwestern France. Durette-Desset (1968) clarified the concept for this species, matching it to the description of *Longistriata wolgaense* Schulz, 1926. The species was initially transferred to *Longistriata* (see Durette-Desset 1968) and then *Boreostrongylus* (see Durette-Desset 1971) before placement within *Carolinensis* (see Durette-Desset 1983). Records of *C. minutus*, or its junior synonyms, indicate a widespread distribution in arvicoline voles (often *Microtus* spp.) across the Palearctic. This distribution extends through western Europe (Feliu et al. 1991; Audebert et al. 2005; Adalid et al. 2021), central and eastern Europe and Russia (Meszaros 1977; Kirillova et al. 2020), into the Levant (Wertheim and Durette-Desset 1975), and eastwards as far as Nepal (Asakawa et al. 1997) and Japan (Ito and Itagaki 2003) (Fig. 1).

Carolinensis minutus has only previously been reported in the United Kingdom (U.K.) on a few occasions. This includes from the Inner Hebrides in Bank Voles [*Myodes glareolus* (Schreber, 1780)] in the 1950s (Thomas 1953), as *L. wolgaense*, and, more recently, in captive-bred Water Voles [*Arvicola terrestris* (Linnaeus, 1758)] released in the London area in 2001 (Mathews et al. 2006). The species has never, to our knowledge, been recorded in the Field Vole [*Microtus agrestis* (Linnaeus, 1761)] in the U.K., even though this is one of the hosts for *C. minutus* in mainland Europe and is also the most common wild mammal in the U.K. (Matthews and Harrower

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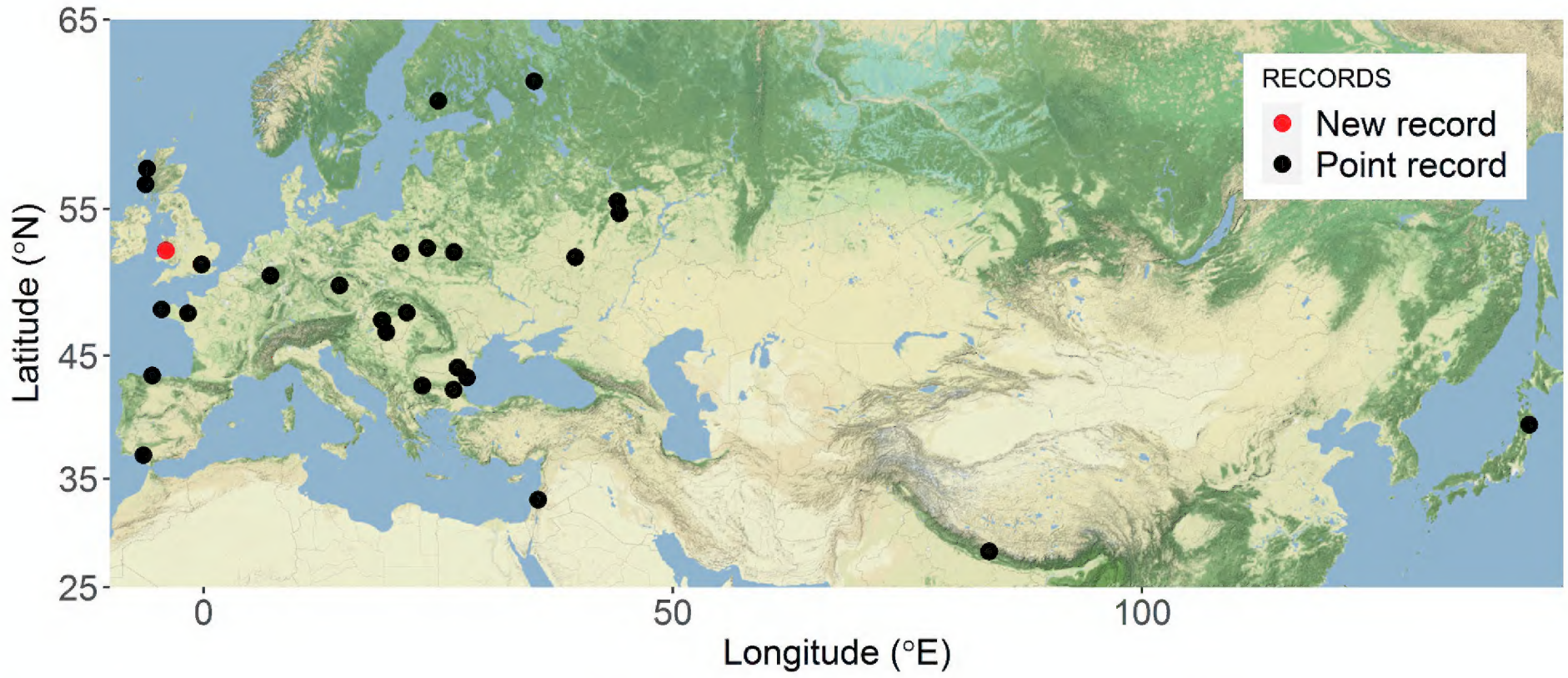

Figure 1. The geographical distribution of *Carolinensis minutus* in the Palearctic. Exact or approximate point records based on information derived from references cited in the main text and from Bugmyrin et al. (2015), Tenora et al. (1980), and Torres et al. (1983). The new record is the first in mainland Britain not associated with the release of captive-bred hosts.

2020). Previous records of heligmosomoids in wild *M. agrestis* in the U.K. are limited to *Heligmosomoides glareoli* Baylis, 1928 and *Heligmosomoides laevis* (Dujardin, 1845) (see Elton 1931; Lewis 1987; Jackson et al. 2014). Elton's record, from Oxfordshire, was of *H. polygyrus sensu* Boulenger (1922) which was later placed as a junior synonym of *H. laevis* (see Durette-Desset 1968, 1971). Jackson et al. (2014) recorded *H. laevis* at low prevalence (2, 3, 4, and 10%) at four sites in the Kielder area, Northumberland [these data being included in the supporting information for Jackson et al. (2014)]. It can also be noted that *H. glareoli* sometimes occurs at other sites in the Kielder area (unpublished observations).

Microtus agrestis is most abundant in meadows, particularly in wet areas and alongside watercourses, and in areas of dense herbaceous cover within forests, but it may occur at lower densities in a range of other habitats (da Luz Mathias et al. 2017). On a number of occasions in early 2014, in mid Wales, *M. agrestis* was observed in a relatively unusual setting for this species, inhabiting underground tunnels of the European Mole (*Talpa europaea* Linnaeus, 1758). One tunnel-inhabiting vole was examined for heligmosomoid parasites, revealing relatively small specimens (<2 mm in length) in the duodenum inconsistent with *Heligmosomoides* and similar to *C. minutus*. Below, the identity of this material is considered.

Methods

A single *Microtus agrestis* (non-scrotal male, weight 19.3g) was captured at Aberystwyth in mid Wales in February 2014 using a humane mole live-trapping tunnel trap with an internal chamber and one-way door (manufacturer not known). The trap was set, during the day, in an area of managed lawn, dug into a mole tunnel of intermediate depth (c. 10cm) and with the turf above placed back in its original position. The trap was recovered, still in daylight, after 4 hours. Following capture, the animal was humanely killed using a U.K. Home Office approved method (terminal overdose of chloroform followed by exsanguination) and the gastrointestinal tract was immediately examined for gastrointestinal parasites. Three heligmosomoid specimens recovered from the duodenum were stored in RNA stabilization solution (RNAlater, ThermoFisher Scientific). Prior to immersion in RNA stabilization solution the specimens were briefly examined by low power microscopy, but not by high power microscopy to avoid compromising their integrity as molecular samples. Work followed ethical procedures at the Institute of Biological, Environmental and Rural Studies, Aberystwyth University.

The material was subject to RNAseq analysis as a control in another study. RNA was initially extracted using the Arcturus PicoPure RNA Isolation Kit (ThermoFisher Scientific). Due to low yields and sample quality only one sample was processed to the sequencing library stage, employing the SMARTer Ultra Low RNA library kit (Clontech). The resulting library was sequenced on a NovaSeq platform (Illumina) yielding c. 53 million 100 bp paired end reads. Fastq files containing reads from the machine run were quality controlled and trimmed using Fastp (Chen et al. 2018) and then used to construct a *de novo* transcriptome with Trinity v. 2.9.1 (Grabherr et al. 2011).

As the size (body length < 2mm) and general morphology of the specimens under low power microscopy had suggested *C. minutus* as a likely identity, National Center for Biotechnology Information (NCBI) databases were searched for *C. minutus* sequences, revealing a single sequence each for internally transcribed spacer (ITS) 1 (GenBank accession number: AY332645.1) and ITS2 (AY333379.1) rRNA. These database sequences were associated with the publication of Audebert et al. (2005)

which listed them from rodents at Brest, France. The *de novo* transcriptome was searched (blastn) for these sequences.

A phylogenetic tree for the concatenated ITS1 and ITS2 sequences of the sample from mid Wales and other representative heligmosomoids was inferred with MEGA X v. 10.0.5 (Kumar et al. 2018) using the maximum likelihood method. Sequences were aligned with ClustalW at MEGA X default settings. For tree building, all positions with missing data or gaps were eliminated (complete deletion) and 1000 bootstrap replicates were run. Initial trees for heuristic searches were selected as those with the best log likelihood when Neighbor-Join and BioNJ algorithms were applied to a pairwise distance matrix estimated by maximum composite likelihood. A General Time Reversible model was employed, and some sites were allowed to be invariable.

A geographical distribution map was plotted with the *R* package *ggmap* (Kahle and Wickham 2013) using a terrain-background stamen map (showing hill shading and natural vegetation colours).

Results

Phylum Nematoda Diesing, 1861

Superfamily Heligmosomoidea Durette-Desset & Chabaud,1993

Family Heligmonellidae Skrjabin & Schikhobalova, 1952

***Carolinensis minutus* (Dujardin, 1845) Travassos, 1937**

New records. UNITED KINGDOM – **Wales** • Ceredigion, Aberystwyth; 52.4095°, −004.0531°; 66 m alt.; 28.II.2014; J.A. Jackson leg.; host: *Microtus agrestis*; GenBank: ON497118 (ITS1), ON497119 (ITS2); 3 specimens rendered to RNA and then to a *de novo* transcriptome via Illumina sequencing and assembly of short reads.

Specimens were recovered from a single Field Vole (*M. agrestis*) inhabiting tunnels constructed by the European Mole (*T. europaea*).

Identification. A blast search of the Welsh *de novo* transcriptome with the available known sequences for *C. minutus* (ITS1 and ITS2 for French material) recovered a predicted transcript corresponding to pre-rRNA. This contained regions with 99.1% identity with the complete ITS1 and 99.3% identity with the complete ITS2 of French *C. minutus*. For the ITS1, the difference was only three one-nucleotide gaps (two in the French sequence and one in the Welsh sequence) and a single SNP (A Wales/C France). For the ITS2, there was a single one-nucleotide gap in the French sequence and a degenerate nucleotide in the French sequence that was consistent with a corresponding leading strand thymine in the Welsh sequence. There was much lower sequence similarity of the Welsh material with other heligmosomoid lineages, including *Heligmosomoides* (see phylogenetic tree in Fig. 2). The sequence discrepancy between French *C. minutus* and the Welsh material is consistent with intraspecific variation and with a determination of *C. minutus* for the latter.

Discussion

The present results are the first record of *Carolinensis minutus* in *Microtus agrestis* in the U.K. and the first time it has been molecularly defined in this locality. Moreover, to our knowledge, this is the first time the species has been recorded in mainland Britain in a naturally occurring host. Whilst *C. minutus* has previously been reported in captive-bred Water Voles released into the wild in mainland Britain (Mathews et al. 2006), the distance of Aberystwyth from the nearest known Water

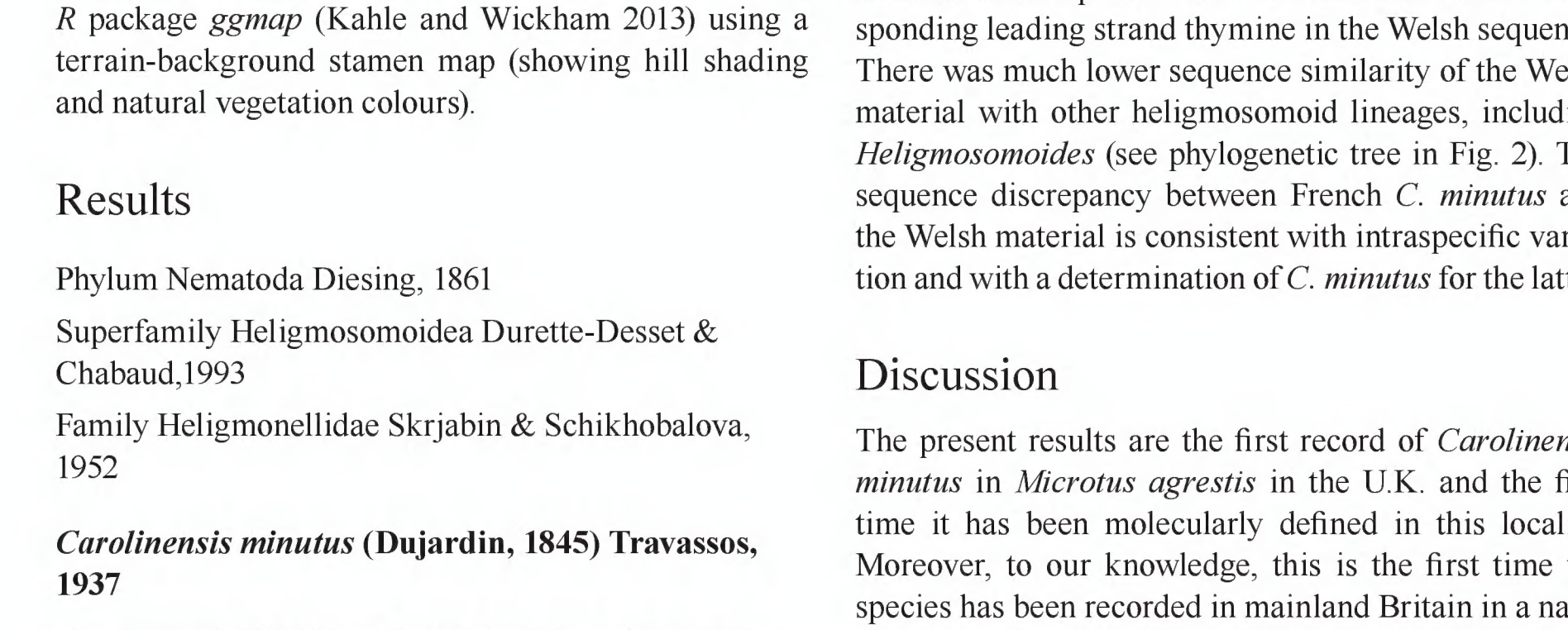

Figure 2. Maximum likelihood phylogenetic tree for concatenated Internally Transcribed Spacer (ITS) 1 and ITS2 sequences in *Carolinensis minutus* in mid Wales and Brest, France and in other representative heligmosomoids. GenBank accession numbers for ITS1 precede the taxon names. The tree with the highest log likelihood is shown, based on 493 positions. Percentage bootstrap support is shown for taxon clusters; branch lengths represent substitutions per site (scale bottom left).

Vole introduction sites, and the timing of such introductions (McGuire and Whitfield 2017), indicate that the parasite population discovered here is unlikely to have originated directly or indirectly from introduced Water Voles. Given the widespread occurrence of *C. minutus* across the Palearctic, it is most likely to have been a long-standing endemic in the mainland U.K. with a rare or patchy distribution. The new record entails that at least three heligmosomoids, *H. laevis*, *H. glareoli*, and *C. minutus*, are now documented to occur in *M. agrestis* in the U.K.

Authors' Contributions

Conceptualization: IF, JJ. Data curation: JJ. Formal analysis: JJ. Funding acquisition: JJ. Investigation: JJ, IF. Methodology: IF. Project administration: JJ. Writing – original draft: JJ, IF. Writing – review and editing: IF, JJ.

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